

ADENOSINETRIPHOSPHATASE OF MYTILUS SPERMATOOZOA.

II. EFFECTS OF SULFHYDRYL REAGENTS, TEMPERATURE AND INORGANIC PYROPHOSPHATE¹

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The enzyme concentrated in the sperm flagellum of the mollusc, *Mytilus edulis*, which splits adenosinetriphosphate (ATP) has been classified as a "true" adenosinetriphosphatase (ATP-ase) (Nelson, 1955). Mohri (1958) has confirmed this in studies on the flagellar ATP-ase of sea urchin sperm. This class of enzyme liberates only one phosphate group from each ATP molecule, even on prolonged incubation. The *Mytilus* sperm enzyme exhibits optimum activity at about pH 8.4 in veronal and glycine buffers, while for sea urchin sperm, pH 8.8 is optimum in Tris and veronal buffers. Magnesium has a pronounced activating effect on both the molluscan and the echinoderm sperm ATP-ase; however, calcium exerts considerably less activation, and, moreover, antagonizes the potentiation due to magnesium. Dilution of filtered-sea-water or isotonic KCl suspensions of *Asterias* and *Mytilus* sperm tails with large volumes of glass-distilled water or 10^{-4} M $MgCl_2$ causes slow precipitation of the tails; this may be accelerated by the addition of small amounts of ATP. However, extruded threads produced from these sperm tail suspensions do not contract; this may be attributed to the lack of continuity of the components—the individual sperm tails (unpublished observation). Salts of heavy metals and other sulfhydryl reagents which serve as spermicidal agents act to halt sperm motility and also interfere with a regulatory mechanism of sperm respiration; low concentrations of inhibitor permit "uncontrolled" acceleration of oxygen consumption, while higher concentrations completely stop O_2 uptake and motility (Barron *et al.*, 1948; MacLeod, 1951). It has also been observed that sodium pyrophosphate, apparently by forming firm complexes with the divalent cations essential for optimal activity, exerts an inhibitory action on sperm ATP-ase (unpublished). The first paper in this series dealt with some of the characteristics of the *Mytilus* sperm tail ATP-ase. The effect of some additional agents (SH-inhibitors, temperature, pyrophosphate) on spermatozoan ATP-ase is considered in the investigations reported here.

MATERIALS AND METHODS

Since it is difficult to induce spawning in the Woods Hole *Mytilus* either by temperature shock or the injection of KCl, it was necessary to obtain the sperm from minces of the gonads. The sperm were harvested and prepared as reported

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previously (Nelson, 1955). "Decapitation" was effected by means of a stainless steel, piston-type homogenizer, and the heads and tails separated by repeated gentle centrifugation ($1000 \times g$ for ten minutes each) in isotonic KCl. The sedimented heads were discarded. The dilution-precipitation effect was exploited in the further isolation and purification of the tails from the pooled supernatant fractions by the addition of at least five volumes of ice-cold $10^{-4} M$ $MgCl_2$. After standing in the refrigerator for 6 to 12 hours, the tails had settled out and the clear supernate was decanted and discarded. The flocculent precipitate was further concentrated by centrifugation at $7000 \times g$ for 10 minutes. The sperm tail concentrate was then taken up in two to four volumes of isotonic ($M/2$) KCl. (All solutions were

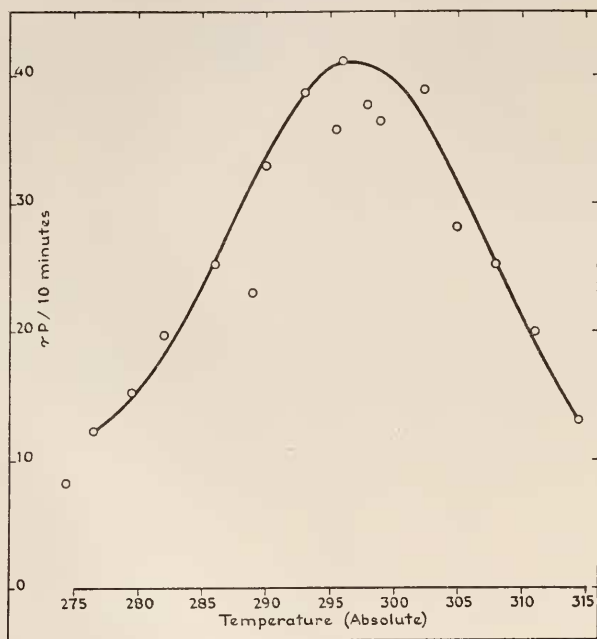


FIGURE 1. Temperature dependence of *Mytilus* sperm tail ATP-ase. Reaction mixture: 0.05 M KCl; 0.04 M Tris buffer, pH 8.6; $10^{-4} M$ $MgCl_2$; $10^{-3} M$ ATP; 0.1 ml. sperm tail suspension; total volume: 1.0 ml. Incubation time, 10 minutes. Ordinate, phosphate liberated in 10 minutes; abscissa, temperature, degrees, Absolute.

made up in de-ionized water.) Since the tail preparation resisted solubilization in mild alkaline and detergent solutions, no further effort was made to extract them and the experiments were performed on the "intact" tails. The reactants, mixed by lateral agitation in 12-ml. Pyrex conical centrifuge tubes, consisted of 0.1 or 0.2 ml. of sperm tail suspension, 0.8 or 0.7 ml. Tris buffer (Sigma), pH 8.6 (0.05 M KCl, $10^{-4} M$ $MgCl_2$, 0.04 M Tris), unless otherwise noted. After the reactants had equilibrated in a thermostat at 24° to $25^\circ C.$, 0.1 ml. $10^{-3} M$ ATP (Sigma disodium salt, neutralized with NaOH to bromthymol blue endpoint) was added and the mixture allowed to incubate for ten minutes. Addition of one ml. of ice-cold 10% trichloroacetic acid terminated the reaction. The precipitate was

removed by centrifugation and the entire supernate analyzed for orthophosphate by the microcolorimetric method of Taussky and Shorr (1953). Optical density was measured in a Coleman, Junior spectrophotometer at a wave-length of 660 millimicrons. Protein content of the sperm tail samples was estimated by a modification of the method described by Nielsen (1958).

RESULTS

Temperature-dependence of flagellar ATP-ase. Duplicate determinations of the enzyme activity at various temperatures over a range from 1° to 41.5° C. show a fairly constant increase in rate up to about 20° C., virtually a doubling for each

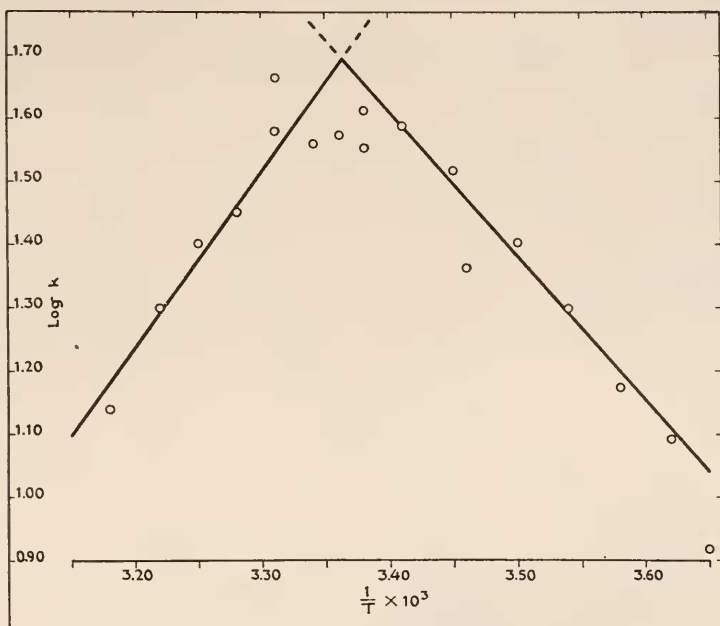


FIGURE 2. Temperature dependence of Mytilus sperm tail ATP-ase. Arrhenius plot of data in Figure 1. Ordinate, logarithm of rate of phosphate liberated; abscissa, reciprocal of temperature, $1/\text{degrees, Absolute}$.

ten-degree rise ($Q_{10} = 2$) (Figure 1). Between 20° and 30°, the rate of dephosphorylation levels off and then declines fairly uniformly. From the slope of the Arrhenius plot (Fig. 2), the activation energy of the reaction was calculated to be $-10,450$ cal./degree/mole. Since these determinations have been made on crude preparations, on "whole" tail suspensions, rather than purified enzyme extracts, this finding suggests that the broad temperature range of maximum enzyme action may simulate the situation which occurs during natural spawning. This coincidence may be of significance in that when associated with other factors (chemotactic, antigenic, etc.; cf. review by Rothschild, 1956) which may operate to assure maximum fertilization, optimum swimming activity of the spermatozoa may further serve

to increase the number of effective sperm "collisions" with activatable eggs. The dependence of spermatozoan motility on utilization of ATP has been established (Mann, 1945; Rothschild and Mann, 1950; Nelson, 1958a).

Effect of sodium pyrophosphate on sperm ATP-ase

Presence of an inorganic pyrophosphatase in flagella. Preliminary observations indicated that when the sperm tail incubation medium contained ATP and sodium pyrophosphate (NaPP) in the ratio of 1.6:1, the amount of inorganic phosphate

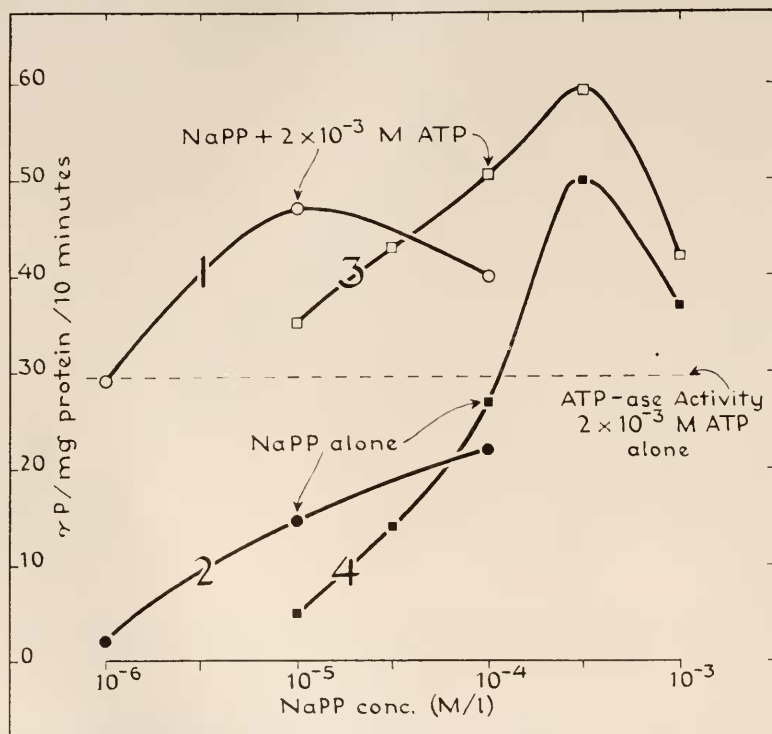


FIGURE 3. ATP-ase and IPP-ase activity of isolated *Mytilus* sperm tails. Reaction mixture: 0.05 *M* KCl; 0.04 Tris buffer, pH 8.6; 5×10^{-6} *M* $MgCl_2$ (dotted line and Curve 1, open circles, and Curve 2, closed circles) or 10^{-4} *M* $MgCl_2$ (Curve 3, open squares and Curve 4, closed squares); 2×10^{-3} *M* ATP (Curves 1 and 3 and dotted line); varying concentrations of NaPP; 0.1 ml. sperm tail suspension. Total volume, 1.0 ml. Incubation: 10 minutes, 24.5° C. Ordinate, γ phosphate liberated/mg. protein/10 minutes, abscissa NaPP concentration (M/liter).

liberated was 88% of the control (no NaPP). When the molar ratio of ATP to NaPP was 0.8:1, only 57% of the control activity was found. Tentatively, this was interpreted as an inhibition caused by the removal of the activating divalent cations through their chelation by the pyrophosphate. Verification was deferred until the present.

The sperm tail suspension, incubated for 10 minutes at 24.5° C. in 2×10^{-3} *M*

ATP and $5 \times 10^{-5} M$ $MgCl_2$, splits off about 30γ phosphate per mg. of sperm tail protein (dashed line, Fig. 3). When varying amounts of NaPP are added to this mixture, the increase in phosphate liberation evidently depends on the relative concentrations of NaPP and $MgCl_2$ in the medium. Curve 1 (open circles) shows that a peak of activity is reached at $10^{-5} M$ NaPP in the ATP-containing medium; while at a somewhat higher $MgCl_2$ ($10^{-4} M$) concentration, curves 3 and 4 (open

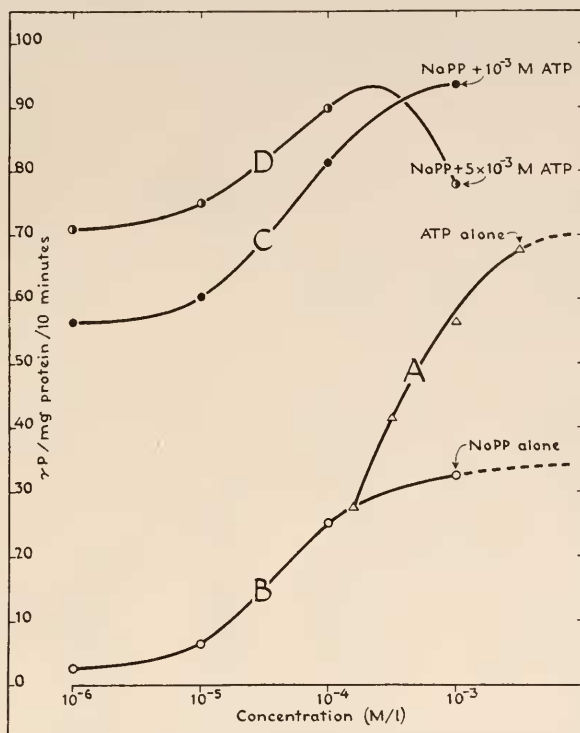


FIGURE 4. ATP-ase and IPP-ase activity of isolated *Mytilus* sperm tails. Reaction mixture: $0.05 M$ KCl; $0.04 M$ Tris buffer, pH 8.6; $5 \times 10^{-5} M$ $MgCl_2$; varying concentration of NaPP (Curves B, C, D, open, closed and half circles, respectively); varying concentrations of ATP (Curve A, open triangles); $10^{-3} M$ ATP (Curve C), $5 \times 10^{-3} M$ ATP (Curve D); 0.1 ml. sperm tail suspension (more concentrated than in previous figures). Total volume 1.0 ml. Incubation: 10 minutes, $24^\circ C$. Ordinate, γ phosphate liberated/mg. protein/ 10 minutes. Abscissa, concentration of phosphate ester (NaPP—curves B, C, D; ATP—curve A).

and closed squares) exhibit peaks of activity at $5 \times 10^{-4} M$ NaPP, both in the presence and absence of ATP.

It is apparent from these results that (i) the sperm tail preparations exhibit an active inorganic pyrophosphatase (IPP-ase); (ii) that this enzyme activity is additive to that of the ATP-ase; (iii) that above an optimum substrate (NaPP) concentration there is a depression of the IPP-ase; (iv) that this optimum may be related to the magnesium concentration; and (v) that the presence of ATP may even aggravate the depression of IPP-ase activity under certain circumstances.

These observations were confirmed and the situation elucidated with a fresh sperm tail preparation. The conditions of the experiment were adjusted by doubling the MgCl_2 concentration (from $5 \times 10^{-5} M$ to $10^{-4} M \text{MgCl}_2$), by making up the ATP in the Tris buffer-KCl- MgCl_2 medium to maintain the total $[\text{MgCl}_2]$ constant while varying the $[\text{ATP}]$ and by increasing the sperm tail content in the incubation mixture (Fig. 4). The ATP-ase activity curve (A) approaches a maximum velocity of 70γ phosphate/mg. protein/10 min. with increasing substrate concentration. The IPP-ase activity approaches a maximum velocity about half that of the ATP-ase (34.5γ phosphate/mg./protein/10 min., Curve B). When, as shown in Curve C, increasing amounts of NaPP are added to the sperm tail incubation

TABLE I

1 Substrate concentration (M/liter)			2 Phosphate liberated (γ)			3 Difference	
ATP	NaPP	ΔP + NaPP	Observed or extra- polated	If ATP	If NaPP	γ Phosphate	
			a	b	c	a - b	a - c
0.001	0.000001	0.001001	56 2.5	56	32.5	0	23.5
			56	56.5	32.5	-0.5	23.5
	0.00001	0.00101	6.5				
	0.0001	0.0011	60 25	57	32.5	3	27.5
			81	60	33	21	48
	0.001	0.002	32.5 93.5				
0.005	0.005001		67.5 71	62 67.5	33.2 34.5*	31.5 0	60.3 33
			75	67.5	34.5*	3.5	36.5
	0.00501		75	67.5	34.5*	7.5	41.5
	0.0051		90	68	34.5*	22	55.5
	0.006		78	69*	34.5*	9	43.5

* V_{max} calculated from Lineweaver-Burk plot (Fig. 5).

ATP-ase and IPP-ase activity. Reaction mixture: $0.05 M$ KCl, $0.04 M$ Tris, pH 8.6, $5 \times 10^{-5} M$ MgCl_2 ; 0.1 ml. sperm suspension; and varying concentrations of ATP and NaPP. Total volume, 1.0 ml. Incubation conditions: 10 minutes, 24.5°C . Enzyme activity = γ phosphate split/mg. protein/10 minutes incubation.

medium containing $10^{-3} M$ ATP, the activity is increased by 50 to 60γ phosphate at all concentrations of NaPP, so that activity curves B and C appear parallel. However, when the medium contains $5 \times 10^{-3} M$ ATP plus increments of NaPP (Curve D), the initial increase in rate of dephosphorylation approximates that attributable to the increase in ATP concentrations. Subsequently the rate increases slowly, approaching the maximum attained in Curve C, although at about $\frac{1}{5}$ the NaPP concentration. Thereafter the velocity of the enzymic action declines rapidly.

The data summarized in Table I support the conclusion that two distinct enzymes are involved in the dephosphorylation of ATP and NaPP. If all the hy-

hydrolyzable phosphate were present as ATP, in Curve C of Figure 4, the maximum amount of phosphate split under the conditions of this experiment (enzyme concentration limiting) could not greatly exceed 62 γ instead of 93.5 γ (column 2); if the total source of P were NaPP, then the maximum P liberated probably could be only 33.2 γ . Similarly, if total hydrolyzable P in Curve D were present in the

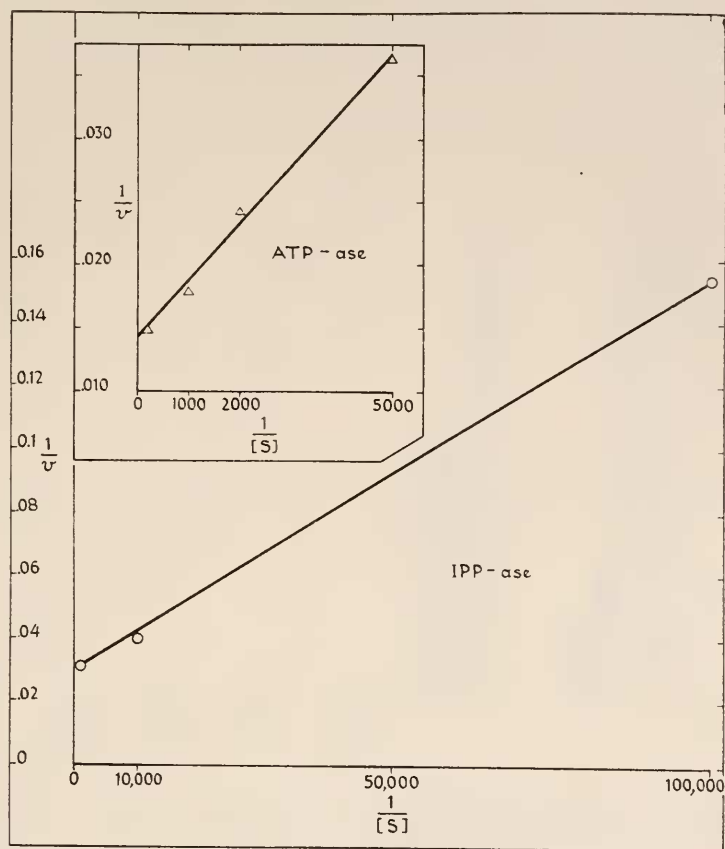


FIGURE 5. ATP-ase and IPP-ase activity of isolated *Mytilus* sperm tails. Lineweaver-Burk plot. Reaction mixture: 0.05 M KCl; 0.04 M Tris buffer, pH 8.6; 5×10^{-5} M $MgCl_2$, 0.1 ml. sperm tail suspension; varying concentrations of substrate; total 1.0 ml. Incubation: 10 minutes, 24° C. Open circles—IPP-ase; inset-open triangles—ATP-ase. Ordinate, reciprocal rate of phosphate liberation/mg. protein/10 minutes; abscissa, reciprocal of substrate concentration. $K_{M-IPP-ase} = 4.3 \times 10^{-5}$; $V_{max-IPP-ase} = 34.5$. $K_{M-ATP-ase} = 3.1 \times 10^{-4}$; $V_{max-ATP-ase} = 70$.

form of ATP, the maximum liberated P would not exceed 69 γ , or if present in the form of NaPP, probably would not be in excess of 34.5 γ . It is further evident that when both sperm tail content and $MgCl_2$ concentration in the incubation mixture are increased, in contrast to the situation in Figure 2, the NaPP does not interfere with itself as suggested by the optimum in curve 4, Figure 2, but that addition of

ATP at high enough concentration duplicates this phenomenon (Curve D, Fig. 3). Mohri (1958) and Nelson (1955) have shown the distinctive magnesium activation of the sperm tail ATP-ase. Evidence from the literature suggests that, with one notable exception cited in Discussion, regardless of enzyme source, inorganic pyrophosphatase activity is generally limited by the magnesium content of the medium. In this respect, the flagellar IPP-ase is not unique. The mutual dependence of these enzymes on adequate magnesium ion suggests the validity of the original concept that by chelation, NaPP competes with ATP for the magnesium ions and thereby could exert an inhibitory influence on the ATP-ase activity. The

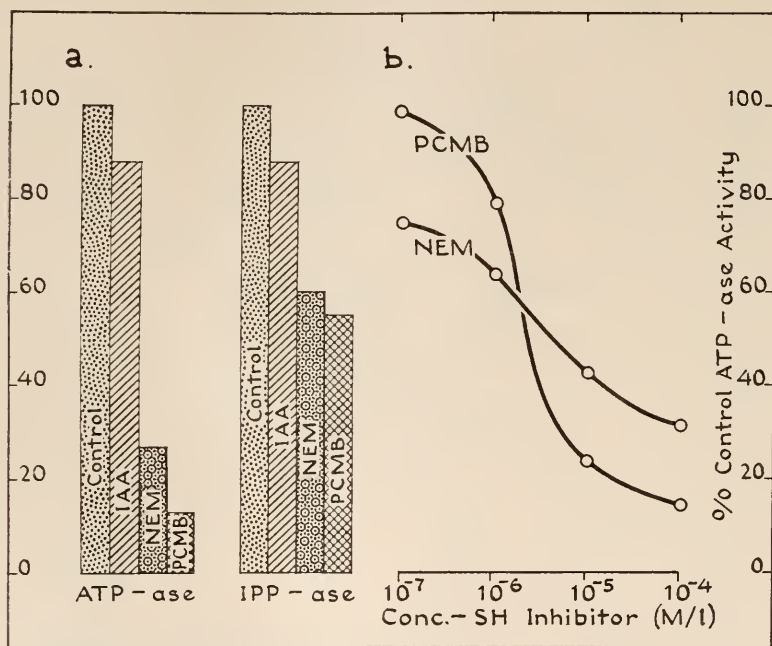


FIGURE 6. Effect of sulfhydryl inhibitors on *Mytilus* sperm tail enzymes. a. Effect of 10^{-4} M SH-inhibitor: Ordinate, per cent of control enzyme activity; abscissa, bar 1, control; bar 2, moniodoacetate; bar 3, N-ethyl maleimide; bar 4, p-chloromercuribenzoate. b. Curves showing effect of varying concentrations of N-ethyl maleimide and p-chloromercuribenzoate on sperm tail ATP-ase. Ordinate, per cent of control ATP-ase (no inhibitor); abscissa, concentration of SH-inhibitor (M/liter). Incubation: 10 minutes, 24.5° C.

possible significance for sperm motility of this mutual interaction of substrates and enzyme activities will be considered later.

When the Lineweaver-Burk (1934) analysis is applied to these unpurified preparations, the Michaelis constants K_m , and the maximum velocities V_{max} of the respective enzymatic reactions may be determined graphically (Fig. 5). For the IPP-ase, K_m is 4.3×10^{-5} , and V_{max} is 34.5γ P/mg. protein/10 min., while for ATP-ase K_m is 3.1×10^{-4} , and V_{max} is 70γ P/mg. protein/10 min. (K_m for erythrocyte IPP-ase equals 5.4×10^{-4} according to Bloch-Frankenthal, 1954; and K_m for skeletal muscle ATP-ase equals 1×10^{-4} to 3×10^{-4} , according to Watanabe

et al., 1952.) Under these conditions, Q_p of the ATP-ase equals 300 as compared to 150 for the bull sperm ATP-ase (Nelson, 1954), and would probably be somewhat higher for purified sperm tail extracts. Further evidence substantiating the belief that two separate enzymes are involved derives from comparison of the effects of sulfhydryl reagents on the dephosphorylation of the two substrates.

Sulfhydryl inhibition of flagellar ATP-ase and IPP-ase. The different sulfhydryl inhibitors caused varying degrees of inhibition of the enzymes. ATP-ase is more sensitive than IPP-ase to the action of both N-ethyl maleimide and p-chloromercuribenzoate at an inhibitor concentration of 10^{-4} M, while sodium monoiodacetate inhibits both enzymes only very slightly (bar graphs, Fig. 6a). (HgCl_2 at the same concentration completely inhibits the ATP-ase.) P-chloromercuribenzoate, a mercaptide-forming agent, is more effective at higher concentrations, while N-ethyl maleimide, an alkylating agent, is relatively more potent at lower concentrations in inhibiting the sperm tail ATP-ase (Fig. 6b). This may reflect the fact that while N-ethyl maleimide is a specific sulfhydryl inhibitor, p-chloromercuribenzoate at the higher concentrations may be combining with other reactive and essential sites on the protein side-chains in addition to sulfhydryls (Boyer, 1959). Unfortunately, no attempt was made at the time to reactivate the enzymes by treatment with sulfhydryl compounds such as BAL (2, 3 dimercapto-propanol), glutathione or cysteine.

DISCUSSION

The studies on the temperature dependence and the effects of sulfhydryl inhibitors are relatively straightforward. Kielley and Bradley (1956) reported that with calcium as activator, when approximately one-half of the sulfhydryl groups of myosin are titrated with either p-chloromercuribenzoate or N-ethyl maleimide a marked increase in ATP-ase activity occurs. Other distinguishing features of the myosin- and actomyosin-ATP-ase and sperm tail ATP-ase have already been considered (Nelson, 1955). Under the conditions of the present experiments, the flagella again differ from the muscle ATP-ases in exhibiting none of the sulfhydryl reagent activation; whether this is characteristic only of the purified enzyme or of Ca-activated ATP-ase, remains to be investigated.

Of particular interest has been the finding that the sperm tails actively dephosphorylate pyrophosphate. Mohri (1958) concludes that Hemicentrotus sperm tails which hydrolyze ATP are enzymatically inactive to a number of other phosphate esters among which he includes inorganic pyrophosphate. Heppel and Hilmoie (1951) describe an inorganic pyrophosphatase in bull seminal plasma, with a sharp optimum at pH 8.6. The bull seminal IPP-ase has nearly maximal activity in the absence of magnesium, while firefly (McElroy *et al.*, 1951, 1953) and yeast IPP-ase have an absolute requirement for Mg^{++} , as apparently does that of Mytilus sperm tail (although this requires further study). The bull seminal IPP-ase, unlike that of yeast and the sperm flagellum, shows no inhibition by increased substrate. Since metaphosphate, which also forms firm complexes with Mg^{++} , but is not acted on by the enzyme, inhibits yeast IPP-ase in the same concentration range as pyrophosphate, Heppel and Hilmoie (*loc. cit.*) attribute inhibition by high pyrophosphate to Mg^{++} binding. This interpretation is substantiated by the present studies involving the combined action of ATP and NaPP. Moreover, inorganic pyro-

phosphate inhibits magnesium-activated myofibrillar ATP-ase when the total concentration of ATP and NaPP exceeds that of the $MgCl_2$ (Perry and Grey, 1956), and decreases the light scattering of actomyosin solution in the presence of magnesium (Tonomura *et al.*, 1952) even though the pyrophosphate is not split. A number of the nucleoside triphosphates also possess this property of modifying, or interfering with the myosin or actomyosin interaction with ATP, and so the behavior of inorganic pyrophosphate is not unique in this respect (Hasselbach, 1956). However, when considered in conjunction with the activity of the enzyme inorganic pyrophosphatase, this substance assumes peculiar significance and invites speculation as to its possible role as a regulator of a specific cellular energetic reaction. To cite several instances, in addition to vertebrate muscle, for which inorganic pyrophosphate may also serve as an extractant, both the substrate and enzyme may be involved in such diverse activities as firefly luminescence and insect flight. McElroy and his co-workers (1953) report that the decrease in light intensity after mixing ATP, luciferin, luciferase, O_2 , and Mg^{++} is due to the formation of an inactive complex of luciferase which depends on magnesium and a second protein, namely, IPP-ase. Addition of pyrophosphate causes a sharp increase in the light, but inhibitors of the pyrophosphatase (Mn^{++} , Ca^{++} , F^-) must be added to prevent the rapid decay of the high light intensity obtained with the pyrophosphate. (However, iodoacetate, even at concentrations of 10^{-3} and 10^{-2} M does not inhibit this pyrophosphatase.) Gilmour and Calaby (1953) suggest the possibility that pyrophosphate hydrolysis may have some importance as a source of energy for cellular processes, since locust thoracic muscle pyrophosphatase is three times higher than that of femoral muscle, and also refer to the report that the heat of hydrolysis of pyrophosphate is approximately 9000 cal./mole (Ohlmeier and Shatas, 1952). It is unlikely that such an enzymatic reaction is without physiological consequence. An interpretation in harmony with the wide variety of evidences of pyrophosphate involvement in cellular processes may be deduced from evidence that pyrophosphate is one of the naturally occurring "relaxing" or plasticizing factors. Pyrophosphate duplicates the softening effect of ATP in glycerinated muscle fibers and sperm flagella, so-called "cell models" (Bishop, 1958a). Magnesium is essential for the production and maintenance of the extensibility and plasticity of glycerol-extracted muscle (Bozler, 1954a), by physiological concentrations both of ATP and of NaPP. Bozler (1954b) proposed that relaxation is caused by the inactivation of bound calcium and that the relaxed state is due to the formation of an enzymatically inactive protein-ATP-Mg complex. He suggests (p. 157) that "the effect of ATP depends on a balance between two antagonistic actions, contraction, which is caused by the breakdown of ATP, and a softening action like that caused by PP. Whether contraction or relaxation occurs then depends on which of these effects predominates."

[“Elementary processes in muscle action,” Morales *et al.* (1955) should be consulted for a review of the actions of two other naturally occurring modifying factors, myokinase and the system ATP-creatine-transphosphorylase + creatine phosphate, as well as EDTA, and the features held in common by these very different substances.] Bishop (1958b) believes that one or more of the relaxing systems may play roles in sperm model “motility,” since glycerinated sperm twitch repetitively on addition of ATP, ADP, or ITP, while pyrophosphate increases the amplitude of the twitch induced by these substances in rodent sperm. However,

these models are capable of very little, if any, progressive movement, the rate of oscillation is usually slower than that of fresh sperm, the wave is not propagated, and the movements are occasionally restricted to one or another portion of the tail. In this connection, it is worth noting that inorganic pyrophosphatase is a water-soluble enzyme, and as such is one of several components extracted upon glycerination (Nelson, 1959). Isolated fresh sperm tails, also, may oscillate or twitch (Gray, 1958), and so the control or regulation of the undulatory flagellation is most likely an autonomous function of the flagellum itself. Initiation of motile activity may depend on "extraneous" excitatory factors, *e.g.*, the so-called dilution effect, hormones, partial pressure of O_2 , or CO_2 , etc. (*cf.* Mann, 1954). But once the sperm is activated, propagated contraction waves progress down each of the nine outer longitudinal fibers in sequence in such a fashion that while one fiber is contracting, the ones opposite are plastic, undergoing relaxation-activation cycles which immediately succeed their own contraction waves (Nelson, 1958b). This sequence could impart the spiral twist observed in the undulatory wave and perhaps as well the spiral thrust which characterizes the progressive movement of the sperm. In cytochemical terms this may be visualized as follows: The contractile protein-Mg-ATP complex is the condition of the "active" state. Upon contraction, a rigor-like state might ensue, except that pyrophosphate then combines with the contracted fiber through Mg^{++} , inducing the relaxation phase; but since NaPP cannot replace the contraction-inducing property of ATP, and the kinetics of dephosphorylation suggest that the two substances are mutually inhibitory at physiological levels, the NaPP "block" must be removed from the spatial proximity of the contractile site. This may be effected enzymatically by the Mg^{++} -activated IPP-ase. Now, locally resynthesized ATP may recombine with the protein in complex with Mg^{++} released from combination with pyrophosphate. This type of contraction-relaxation cycle, resembling a spatially compact reciprocating mechanism, obviates the necessity for invoking "long-range" migrations of reactants. The highly speculative nature of this scheme may eventually be resolved when the mechanism and site of resynthesis of the inorganic pyrophosphate are discovered, although this should not be an insurmountable objection to the working hypothesis, since both pyrophosphate and pyrophosphatase apparently occur in a wide variety of biological systems (*cf.* Bloch-Frankenthal, 1954).

SUMMARY

1. *Mytilus* sperm tail ATP-ase temperature coefficient (Q_{10}) = 2; temperature optimum occurs in the range between 20° and 30° C.
2. The sperm tails actively dephosphorylate sodium pyrophosphate (NaPP) as well as ATP. Two separate enzymes are involved, which together with their substrates apparently compete for the magnesium ions in the medium.
3. $K_{M-ATP-ase} = 3.1 \times 10^{-4}$; $K_{M-IPP-ase} = 4.3 \times 10^{-5}$.
4. In low concentrations of ATP and NaPP, the amount of inorganic phosphate liberated is additive, while at higher concentrations, inhibition occurs.
5. ATP-ase is more sensitive than IPP-ase to sulfhydryl inhibition, although iodoacetate has only slight effect on both enzymes.
6. An hypothesis is proposed that regulation of the undulatory flagellar wave primarily resides within the flagellum itself, and is a function of the reciprocal

activity of two enzymes, ATP-ase in the contraction phase, and IPP-ase in the relaxation phase.

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